Vol. LXXXII, No. 3 June, 1942

THE
BIOLOGICAL BULLETIN
PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MECHANISM FOR SYNCHRONOUS SPAWNING IN HYDRACTINIA AND PENNARIA

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INTRODUCTION

Synchronous spawning of all individuals in one neighborhood at a particular time of day occurs in various Hydrozoa. It has been noted in *Pennaria* and *Clava* by Beckwith (1909), in *Halyclistis* and *Sertularia* by the Teissiers (1927), and in *Hydractinia* by various workers. These are all colonial species, and the sexual individuals are sessile. Moreover, only one sex is normally represented in each colony, and male and female colonies may live at some distance from each other. If all the eggs and sperm are discharged at the same moment in a given mud flat or tide pool, the chance of survival of the genus is greatly enhanced. How this happens is the subject of the present study.

There have been conflicting reports as to the regular spawning time of *Hydractinia*. Bunting (1894) observed it to be between 9.30 and 10.30 P.M., Teissier and Teissier (1927) between 7 and 8 A.M. C. W. Hargitt (1911) found spawning to occur at various times of the day but most frequently in the early morning. My own observations showed that *Hydractinia* in a state of nature always spawns shortly after sunrise. All but the Teissiers worked at Woods Hole.

Reports from many observers are unanimous that *Pennaria* sheds its eggs and sperm normally several hours after sunset (G. T. Hargitt, 1900; Baker, 1936).

Attempts to force both these species to spawn at any desired hour of day or night have now proved successful, and have opened an approach to the problem of the nature of the spawning mechanism. Reasons can be offered for the conflicting reports on *Hydractinia* and for the difference in preferred spawning time between *Hydractinia* and *Pennaria*.

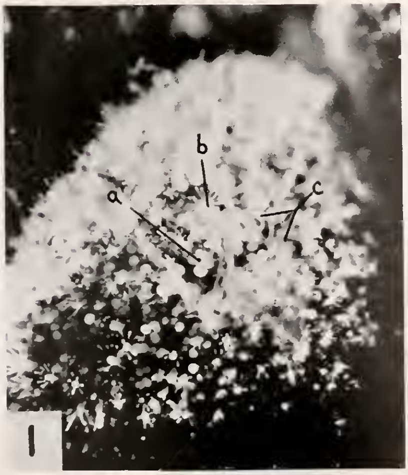
METHODS OF CONTROLLING SPAWNING

Hydractinia echinata Fleming is in good breeding condition at Woods Hole in June and July. The colonies, growing on shells inhabited by hermit crabs, may be kept in open dishes of sea water and will shed eggs or sperm copiously once a day for a week before becoming exhausted.

It was first noted that *Hydractinia*'s sunrise spawning could be delayed indefinitely by leaving the colonies in the dark of a desk drawer or loose box. When they were returned to the light they spawned in about an hour, with some variation in time according to the condition of the colonies.

Colonies continuously illuminated during the night from an ordinary reading lamp omitted the normal spawning at sunrise, and would not spawn at any later time until they had first been darkened for a while and then restored to light.

In practice, spawning was produced in *Hydractinia* at will by (1) leaving the colonies in running sea water under a glowing 100-watt light bulb from the time of collection until needed; (2) then placing them in the dark for from one to several hours; (3) then exposing them to daylight or a desk lamp, at which time the colonies could be examined with a hand lens and segregated according to sex. Practically all male colonies in prime condition would spawn 50 minutes after the moment of re-illumination (Fig. 1), female colonies five minutes later (Fig. 2).



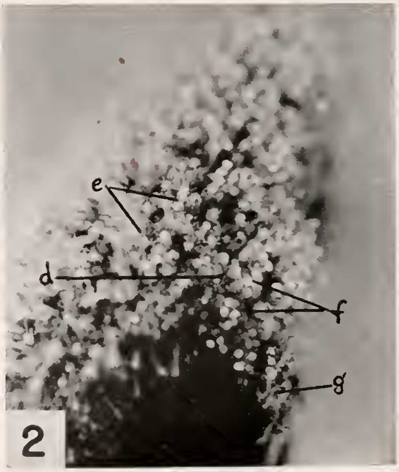
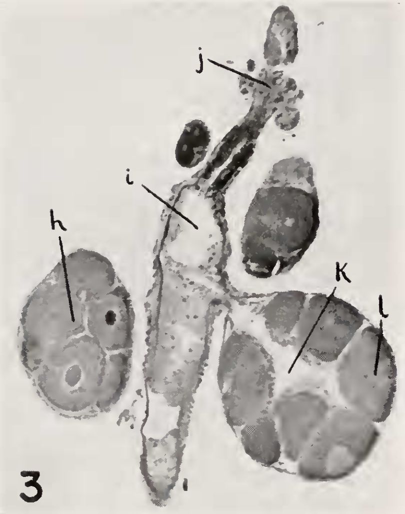
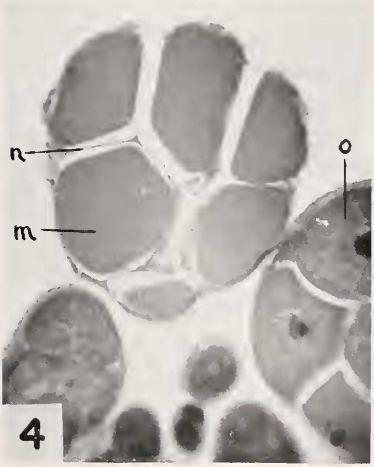


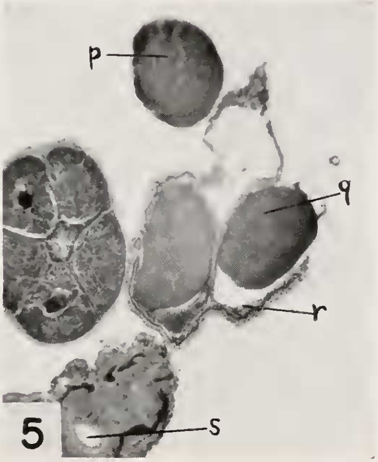
PLATE I

FIG. 1. Male colony of *Hydractinia* just starting to shed sperm. (*a*) Gonozoöid bearing two white ripe gonophores and two translucent unripe gonophores; (*b*) feeding polyp; (*c*) spiral streams of sperm from ruptured gonophores. Toward lower right, a foggy area full of dispersing sperm. About 6 ×.

FIG. 2. Female colony of *Hydractinia* just starting to shed eggs. (*d*) Gonophores containing ripe unshed eggs; (*e*) feeding polyps; (*f*) clusters of eggs just shed; (*g*) stinging polyps around lip of snail shell. About 6 ×.







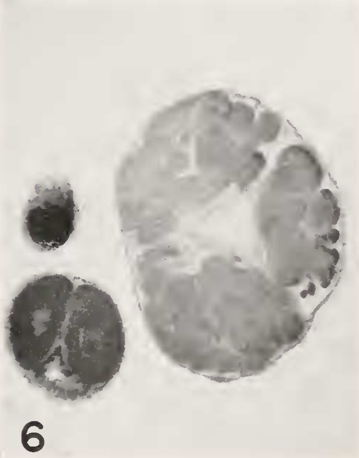


PLATE II

FIG. 3. Section of *Hydractinia* gonozoöid 45 minutes before spawning. (*h*) Gonophore containing unripe eggs; (*i*) gastrovascular cavity of gonozoöid; (*j*) distal end of gonozoöid with nematocysts; (*k*) gastrodermal spadix of ripening gonophore; (*l*) peripherally placed enlarged germinal vesicle of ripening egg. About 60 ×.

FIG. 4. Section of *Hydractinia* female gonophore just before shedding. (*m*) Mature egg cell; (*n*) egg follicle; (*o*) immature oöcyte in a neighboring gonophore. About 100 ×.

FIG. 5. Section of *Hydractinia* female gonophore preserved during the act of spawning. (*p*) Discharged egg; (*q*) egg emerging; (*r*) collapsing wall of gonophore, showing outer epidermis and inner musculo-epithelium; (*s*) gastrovascular cavity of parent gonozoöid. About 100 ×.

FIG. 6. Section showing three *Hydractinia* eggs in late cleavage stages still within the gonophore. Maturation induced by dark and light treatment, shedding inhibited by calcium-free sea water. About 100 ×.

In *Pennaria tiarella* Ayres, both uninterrupted illumination and uninterrupted darkness were likewise found to inhibit the normal evening spawning, as had earlier been suggested by Baker (1936). But after a prolonged period in the dark, spawning took place 10 to 14 hours after return to the light, irrespective of the time of day. The mechanism seems similar, but *Pennaria* reacts far more slowly to light than *Hydractinia.*

Since laboratory aquaria may be in quite dark corners, and since the turning on of lights in the laboratory may precipitate the shedding reactions, it is not surprising that earlier investigators should have reported synchronous spawning of *Hydractinia* at various times of the day. The suggestion of Bunting (1894) that heat and cold control the spawning of *Hydractinia* has not been confirmed, and her experiments in this direction are invalidated by the fact that storing the colonies in a (dark) icebox and later removing them to room temperature (and light) will produce the same result whether the icebox be warm or cold.

Maturation Phenomena

Early accounts of maturation in *Hydractinia* eggs were incomplete or fallacious because material was not collected at the right time of day. Beckwith (1914) was the first to report the cytological details, from study of a quantity of material preserved at dawn. The present investigation corroborates her findings.

Whether spawning be the normal sunrise event or whether it be produced at another time by control of the lighting, the hour following re-illumination and prior to shedding is occupied in maturation. Since the preceding spawning period, the germinal vesicle has enlarged, become sharply granular and moved from a central position to the distal cell wall (Fig. 3), and it now becomes smaller and hazy, disappearing about half an hour after the light stimulus. The first polar body is given off at the same distal point in 45 minutes, the second polar body ten minutes later. The first polar body often divides. At this time, 55 minutes from the time of re-illumination in most material, the wall of the gonophore ruptures (Fig. 4) and the eggs come oozing out (Fig. 5).

Each new egg is covered by a highly transparent but radially striated jelly, which has not been mentioned by previous workers. The jelly swells considerably on contact with sea water, pulling the polar bodies away from the egg surface and pushing the egg away from the collapsing gonophore. Very soon the polar bodies are so far from the egg that they are brushed off by the least disturbance, and lost.

Dissection experiments show that the process of maturation is initiated solely by the action of light on the oöcyte itself, and not by any general action on the colony or the individual. Single gonozoöids isolated from either male or female colonies lived well for five days in watch glasses of sea water, and responded either to the succession of night and day by normal shedding at sunrise, or to controlled illumination by shedding when experimentally planned. Single gonophores isolated from the gonozoöids did likewise, although it was not possible to separate in advance those which would shed from those that would not. In practice, a batch was made up by collecting the ripest-looking single gonophore from each of a number of very well developed gonozoöids; depending on the actual ripeness of the parent colony, anywhere from 0 to 100 per cent of the gonophores so selected would shed their eggs after dark-light treatment.

When large numbers of apparently ripe primary oöcytes were dissected out of gonophores selected in this fashion, and these isolated oöcytes were then subjected to the routine of one hour of darkness and one hour of light, some of them could be watched passing through maturation stages according to the expected timetable, and could later undergo fertilization and cleavage. Sperm added before or during maturation were without effect until emergence of the second polar bodies. The proportion of maturing to non-maturing eggs in a large group of these dissected eggs was the same as the proportion in the control group of intact gonophores from the same colony. In other words, light could stimulate maturation in the isolated oöcyte with the same facility that it did in the normal animal.

Preparation by Darkness

The amount of time required in the dark to prepare the *Hydractinia* colonies for shedding is certainly short, although no spawning will take place in continuously illuminated colonies. Eggs can be ripe enough so that only 15 minutes or less in the dark will prepare them for reaction to light, but a longer period is necessary for maximum yield of eggs. In a group of 36 female colonies that were allowed just 15 minutes in the dark, only one shed any eggs after re-illumination. Eighteen of these same colonies were given several more hours in the dark, and all shed heavily following the second re-illumination.

Performance of colonies that were darkened for half an hour was somewhat better. Forty-two female colonies were kept illuminated for 36 hours or more, then darkened for half an hour. Only 12 shed eggs after re-illumination. Darkness for an hour or longer followed by re-illumination usually produces spawning in 100 per cent of prime fresh colonies.

What takes place within the eggs during the dark period is a question. No nuclear changes are discernible in fresh or sectioned material. Until some time after re-illumination, it is not possible to distinguish the eggs that are going to be shed from the large unripe eggs in neighboring gonophores. Perhaps darkness allows the accumulation of light-sensitive substances whose breakdown initiates the maturation processes.

SPAWNING AFTER RE-ILLUMINATION

In a few cases, colonies kept in a box which admitted a crack of light on one side were found to have spawned before return to full daylight, but when colonies were put in complete photographic darkness after prolonged illumination, eggs were never shed in the dark. Ten female colonies were kept in complete darkness for 28½ hours, without shedding a single egg. On removal to light, all ten colonies shed within the hour.

To test the effectiveness of a short dosage of light, 76 female colonies which had been continuously illuminated for 24 hours since they had been collected were divided into two apparently equivalent batches and stored in complete darkness for 4¼ hours. One was to shed after a brief exposure to light, the other after prolonged exposure to light, and the relative effectiveness of the exposures was to be tested by capacity for re-spawning after a second immediately succeeding period of darkness.

Batch A was illuminated for 80 seconds at 1.15 P.M. and restored to the dark. At 3.15 P.M. it was taken from the dark and the heavy spawn of eggs was removed. Left on the desk in daylight, this batch was then expected to shed more eggs in one hour, but only an insignificant several hundred eggs were produced by 5 P.M.

Batch B was left on the desk in full north window daylight from 1.15 to 2.30 P.M. Heavy shedding of eggs took place from 2.10 to 2.15 P.M., and these eggs were removed. From 2.30 until 3.45 P.M. batch B was stored in complete darkness and then placed in the window by the side of batch A. Only a few dozen eggs were produced in the second spawning before 5 P.M.

Batches A and B were kept illuminated overnight and the experiment was repeated the next day. This time batch A was given only 12 seconds of light at 1.15 P.M. Batch B stayed in the light an hour and a quarter. Both batches shed heavily, in approximately equal quantity. As before, the total elapsed time from first darkness to last illumination was the same in the two batches. After the second dark period, batch A shed only several hundred eggs, batch B shed none at all.

Eggs from each different batch were funneled into a standing column in a burette to measure their volume, and a rough estimate of their number was calculated from counts of samples of known volume. Each batch produced well over 6,000 eggs on each day. Batch B was slightly more productive than batch A in the sum of its two sheddings the first day, but there was no significant difference the second day.

It is clear that from 12 to 80 seconds of strong daylight is sufficient stimulus to bring about a heavy shedding of dark-prepared eggs, and that illumination in excess of this is able to bring about the discharge of only a scattering few more eggs. It is not at all essential that the colonies remain in the light during the period from their re-illumination until the time they are shed.

However, a very brief period of illumination depends for its effectiveness on an ample period of previous darkness. On July 18, 50 female colonies which had been continuously illuminated for over 24 hours were stored in the dark for one hour, exposed then to ten seconds of light from a 100-watt bulb two feet away, and then kept in the dark for 2¾ hours. No eggs whatever were shed, but after standing for an hour longer under the light bulb, a heavy spawn resulted. On the following day, 34 of these same colonies, after 13 hours of illumination, were given an hour in the dark, ten seconds of daylight (north window, midday), and 1½ hours in the dark. On removal to daylight again, a moderate shedding of eggs was found, but within the hour twice as many more eggs were shed as a result of the new illumination. No explanation can be offered why a procedure closely similar to that which failed to cause shedding the first day should succeed the following day. But in both cases it is clear that ten seconds of light, which would have been sufficient to precipitate a near-maximum shedding if the colonies had been kept a long time in the dark, was far less effective after only one hour's darkness.

Role of the Jelly Layer in Egg Shedding

Since the eggs are obviously under considerable pressure inside the gonophore during their maturation phase, and since their jelly layer expands while they ooze outward, it was considered whether the jelly was not the immediate agent in rupturing the gonophore and inducing shedding, simply by the absorption of water. Several considerations make this appear unlikely.

Immature eggs are coated with a dense but entirely transparent layer of jelly tightly confined inside a very thin sphere of flattened follicle cells (Fig. 4). When oöcytes with large germinal vesicles are dissected free from the gonophore, the rupture of the follicle-cell layer causes the jelly

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to swell rapidly and it begins to show very delicate radial striations. If the follicle-cell sphere remains unbroken, the jelly remains unswollen for an indefinite period. This indicates that if the swelling of the jelly causes shedding of the egg there must exist also some egg-induced rupture or altered permeability in the follicle-cell layer, if not the gonòphore wall also, allowing the jelly to imbibe water.

But there is evidence that eggs can be shed from the gonophore after destruction of the jelly. Of several treatments which resulted in the dispersal of jelly, the one least harmful to eggs or polyps was immersion in calcium-free sea water. *Hydractinia* can survive without permanent ill effects in Van't Hoff's artificial sea water with $CaCl_2$ omitted for upwards of 14 hours, *Pennaria* for a considerably shorter period.

Hydractinia gonophores containing eggs undergoing maturation, and gonophores containing germinal-vesicle stages, were rinsed in this solution, and the eggs dissected out in it. Some eggs showed very thin and watery jelly, but most showed none at all. *Pennaria* eggs similarly dissected from the medusae in this solution were invariably obtained without jelly, whereas those dissected out in normal sea water had a thick jelly coat best seen in ink suspension. This effect having been observed, efforts were made in *Hydractinia* to disperse the jelly before shedding could start.

Ten female colonies were kept in the dark nine hours and brought to the light, being transferred at once to calcium-free sea water in which they remained one hour. Returned then to normal sea water, they recovered enough to produce a heavy spawn of eggs in another hour and a half, but practically none of these eggs showed any jelly at all. They could be packed together in contact with each other, which cannot be done with ordinary eggs unless the jelly is removed by violent shaking. These jelly-less eggs were mature, and when inseminated they cleaved on schedule.

Calcium Essential for Spawning

If male or female colonies of *Hydractinia* are exposed to the action of calcium-free sea water for three quarters of an hour or longer just prior to the expected shedding time, no spawning occurs in the solution, and a recovery period is necessary in normal sea water before eggs or sperm are released. The corresponding reactions in the more sensitive *Pennaria* are striking.

Pennaria has well-developed medusa-gonophores, in contrast to the degenerate sporosac-gonophores of *Hydractinia*. When *Pennaria* is ready to spawn, the pendant medusae open their bells, and the inner musculo-epithelium of each starts a rhythmic pulsation which is so prolonged and powerful as to accomplish the discharge of the sexual products. When a colony containing many pulsating medusae is transferred to calcium-free sea water, the musculo-epithelium of each medusa is almost instantly paralyzed. Not even the slightest muscular tremor is found after one minute in the solution. Transfer back to normal sea water is followed by prompt recovery, and at the end of a minute or two the medusae are pulsating as strongly as before.

In *Hydractinia,* the inner musculo-epithelium shares in the degenerate development of the whole sporosac (Goette, 1907, Plates V, VI), and is difficult to demonstrate except during the actual process of extrusion of the eggs (Fig. 5). This and the fact that it is not directly exposed to the action of the fluid until the moment of spawning in the sporosac may explain why *Hydractinia* does not show the extreme rapidity of response to lack of calcium exhibited by *Pennaria.*

Since the calcium-free sea water has been shown to have an effect on the jelly layer around the egg, the possibility of inhibition of shedding also through direct injury to the egg must be considered.

Calcium-free sea water apparently does not hinder the darkness-preparation of the oöcytes inside the gonophores. One colony, remaining in this solution 1½ hours in the dark, was transferred to normal sea water in the light, and produced eggs in 65 minutes. Another colony, after one hour in the dark in this solution, was illuminated then for one minute during transfer to normal sea water and darkness, and presently shed eggs in the dark.

The question whether calcium is necessary for the carrying out of the maturation processes and the initiation of them by light has not been put to critical test. However, as proof that the dosage of calcium-free sea water can be adjusted so as to allow successful maturation and yet inhibit shedding, a case of experimentally produced internal fertilization in *Hydractinia* may be cited.

Twelve female colonies were put into calcium-free sea water in the dark for one hour. Next they spent 30 minutes in the light in normal sea water, then an hour in the light in calcium-free sea water again. No eggs having been shed at the end of that time, the gonophores were carefully scrutinized under the microscope with strong light, and the absence of germinal vesicles noted in a number of the eggs. The colonies were left in normal sea water from then on, and a few eggs were extruded in the next two hours. Examination still showing unshed matured eggs in some of the gonophores, sperm were added to the dishes. Figure 6 shows a section through a gonophore which contained three unshed eggs in late cleavage stages, preserved three and a half hours later.

In many hydrozoan genera, such internal development of embryos is normal. Nothing like it ever occurs normally in *Hydractinia.*

Discussion

Influence of light on breeding seasons or spawning reactions is well known in a number of phyla. In most animals the germ cells are well shielded from light and the action is indirect, through the nervous system or in more subtle ways. Even in the translucent ascidians whose spawning is controllable by light (Rose, 1939), some fertilizable eggs can be obtained by dissection at any time of day or night. I cannot cite any other case in which light directly initiates the maturation processes of animals.

One not altogether consistent case among the Chlorophyceae deserves mention. Kater (1929) found that *Chlamydomonas nasuta* underwent cell division regularly and almost exclusively between 9 and 12 P.M. On the other hand Moewus (1933) reports that *Chlamydomonas eugametos* undergoes cell division both in light and dark, but more cell division occurs in regular 12-hour alternations of light and dark than in light. If the cells, lighted for 12 hours, are then kept dark more than 48 hours, all cell divisions cease, even with all other conditions favoring.

Moewus presents some rather remarkable observations on light-controlled sexual activity in *Chlamydomonas.* The gametes never copulate in the dark. When brought into the light, they must be exposed for 30 to 45 minutes before they are ready to copulate. This delay, perhaps representing some sort of physiological maturation, could be reduced by soaking the unlighted gametes in water which had stood for some time in the light with other gametes of the same sexual strain in it, and then been filtered free of them. According to Moewus the soluble effective substances produced by the gametes in the light are highly sex-specific, and those of opposed sex neutralize each other. Other genera of green algae studied by Moewus revealed none of these peculiar reactions to light.

In *Hydractinia* the fact that shedding of eggs occurs only in a few of the large numbers of gonophores in a colony, and the fact that only mature eggs are shed, suggests that the actual shedding process is initiated by some late-maturation change in the eggs themselves. Whether this is a mere increase in volume which ruptures the gonophores, or a chemical stimulation of the musculo-epithelium, or what, is the subject of further experiments now in progress.

SUMMARY

1. Methods are reported for scheduling the shedding of eggs and sperm by *Hydractinia* and *Pennaria* at any desired time of day.

2. Maturation in these animals occurs only after the germ cells have undergone a period of preparation in the darkness, and is initiated by direct action of light on the germ cells. Once initiated by light, maturation can proceed in the dark. Uninterrupted light, or uninterrupted darkness, results in complete cessation of maturation and spawning.

3. In *Hydractinia* maximum shedding requires at least an hour's darkness-preparation, although less time is sufficient for some eggs. Ten seconds of illumination is sufficient for widespread but not maximum initiation of maturation.

4. The hypothesis is advanced that darkness allows the accumulation of light-sensitive substances within the germ cell, whose breakdown initiates the process of maturation. Oöcytes dissected free from the organism can be matured by appropriate dark-light treatment.

5. Shedding of eggs and sperm takes place simultaneously with the completion of the maturation process. The swelling of jelly around the egg is not an important factor in bringing about shedding. Evidence is presented that contraction of the gonophore wall is an important factor.

6. Paralysis of the gonophore's musculo-epithelium by calcium-free sea water inhibits the shedding of mature eggs and sperm. Fertilization and cleavage of eggs within the gonophore have been produced in *Hydractinia* by causing maturation, inhibiting shedding and adding sperm.

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